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Genome-wide association study and heritability estimate for ectopic ureters in Entlebucher mountain dogs

Inaugural-Dissertation

zur Erlangung der Doktorwürde der
Vetsuisse-Fakultät Universität Zürich

vorgelegt von

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von Oberkirch-Mauensee, Luzern

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2019

Für
meine wunderbare Familie

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Summary

An ectopic ureter is a congenital anomaly which may lead to urinary incontinence and even to end-stage kidney disease if surgery is not performed. A genetic component contributes to the development of this anomaly in Entlebucher mountain dogs (EMD). However, its exact nature remains unclear. Using the Illumina CanineHD bead chip, a case-control genome-wide association study was performed to identify SNPs associated with the trait. Six loci on canine chromosomes 3, 17, 27 and 30 were identified with 16 significantly associated SNPs. There was no single outstanding SNP associated with the phenotype and the association signals were not close to known genes involved in human congenital anomalies of the kidney or lower urinary tract. Additional research will be necessary to elucidate the potential role of the associated SNPs in the development of ectopic ureters in the EMD breed.

Keywords: congenital urological malformation, GWAS, urinary tract development, Canis lupus familiaris

Zusammenfassung

Bei ektopischen Harnleitern handelt es sich um eine angeborene Fehlbildung, die zu Harninkontinenz und, insbesondere ohne chirurgische Korrektur, auch zu Nierenversagen führen kann. Beim Entlebucher Sennenhund (ES) sind genetische Faktoren an der Entstehung dieser Fehlbildung beteiligt, der genaue genetische Hintergrund ist aber noch unbekannt. Um mögliche mit der Fehlbildung assoziierte SNPs zu identifizieren, wurde daher mit dem Illumina CanineHD Bead Chip eine genomweite Fall-Kontroll-Assoziationsstudie durchgeführt. Insgesamt wurden 16 signifikant assoziierte SNPs identifiziert, welche sich verteilt auf total sechs Loci auf den Chromosomen 3, 17, 27 und 30 befinden. Unter diesen SNPs hob sich jedoch keiner durch eine deutlich stärkere Assoziation zum Phänotyp hervor. Die assoziierten SNPs lagen zudem nicht in der Nähe von Genen, welche nach heutigem Kenntnisstand die Entstehung von angeborenen Anomalien der Nieren oder des unteren Harnabflusstraktes beim Menschen beeinflussen. Weitere Untersuchungen sind notwendig, um den potenziellen Einfluss der assoziierten SNPs auf die Entstehung von ektopischen Harnleitern beim ES aufzuklären.

Schlüsselworte: Angeborene Harnleiterfehlbildung, GWAS, Harntraktentwicklung, *Canis lupus familiaris*

Genome-wide association study and heritability estimate for ectopic ureters in Entlebucher mountain dogs

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Abstract

An ectopic ureter is a congenital anomaly which may lead to urinary incontinence and without a surgical intervention even to end-stage kidney disease. A genetic component contributes to the development of this anomaly in Entlebucher mountain dogs (EMD). However, its nature remains unclear. Using the Illumina CanineHD bead chip, a case-control genome-wide association study was performed to identify SNPs associated with the trait. Six loci on canine chromosomes 3, 17, 27 and 30 were identified with 16 significantly associated SNPs. There was no single outstanding SNP associated with the phenotype and the association signals were not close to known genes involved in human congenital anomalies of the kidney or lower urinary tract. Additional research will be necessary to elucidate the potential role of the associated genes in the development of ectopic ureters in the EMD breed.

Keywords: congenital urological malformation, GWAS, urinary tract development, *Canis lupus familiaris*, CAKUT, CALUT

Introduction

In dogs, an ectopic ureter (EU) is a rare congenital anomaly in which one or both ureteral orifices are not located at the anatomically correct position at the vesicular trigone of the bladder (Osborne *et al.* 1995). Instead, the ureteral openings can be found in the bladder neck, the urethra or even in the genital system (Dean 1988; Owen 1973). The most common clinical sign of affected dogs is urinary incontinence (Fossum 1997). However, many dogs, especially males, which show a higher prevalence for EU than females in the Entlebucher mountain dog (EMD) (Fritsche *et al*. 2014), are continent for years and may show incontinence only at an advanced age (Holt & Moore 1995; Reichler *et al.* 2012). The sexual dimorphism of the urogenital system might explain this observation (Holt & Moore 1995). Affected dogs are predisposed for ascending infections of the urinary tract including pyelonephritis; they may have or develop hydroureter and hydronephrosis, as well as changes of kidney size or distorted internal kidney architecture (Holt & Moore 1995; Lamb & Gregory 1998; Niesterok 2016). This may result in fatal end-stage kidney disease if no surgery is performed. The EMD is one of the dog breeds that have a higher risk for EU (Fritsche *et al.* 2014). Out of 552 classified EMD nearly half showed intravesicular ectopia, i.e. 130 females and 132 males. One fifth, i.e. 25 females and 84 males, had at least one extravesicular ectopic termination. Urinary incontinence was a complaint in 3% and 27% of them. Hydronephrosis and/or hydroureter was noticed by ultrasonography in 1% and 14% of the intravesicular and extravesicular cases, respectively. In one third of them this was an incidental finding (Fritsche *et al*. 2012). The breed predisposition indicates a genetic background, however exaggerated breeding restrictions to reduce the risk of clinically affected dogs in the offspring are of concern, as the EMD has already a high average inbreeding coefficient around 40% (Schrack *et al.* 2017). Therefore, selection based on genotypes would be desirable, but first attempts to associate five suitable candidate genes, selected from mouse studies (Uetani & Bouchard 2009), to EU in EMD were unsuccessful (North et al. 2009). In humans, congenital anomalies of the kidney and urinary tract (CAKUT) are a genetically heterogeneous group of developmental disorders (syndromic and non-syndromic) with highly varying phenotypes. Even though single gene mutations were shown to cause renal anomalies in mice, the elucidation of CAKUT cases in humans remains difficult (Nicolaou *et al*. 2016).

Genetic heterogeneity, modifier genes, allelic variation in gene expression, epigenetic effects, complex modes of inheritance and environmental effects may hamper the clarification of the genetic basis of sporadic and familial CAKUT (Yosypiv 2012).

Using complex segregation analysis, our group previously showed a genetic background for EU in EMD and the possible involvement of a major gene for the EU-3 phenotype (Fritsche *et al.* 2014), while the evaluation of an X-linked mode of inheritance, which could explain the observed male predominance for extravesicular ectopia was not successful (Fritsche *et al.* 2014). The goal of the present study was to re-estimate the heritability for EU-3 and identify genetic variants associated with EU and candidate genes within chromosomal regions of such variants.

Material & Methods

Ureteric openings were determined for 1421 EMDs born between 1996 and 2016 and registered by official national kennel clubs. The majority of dogs were between six and twelve months old when ultrasonographical screening was performed by authorized private veterinarians and university clinics. This screening method was previously validated by comparison with dissection (Balogh *et al*. 2015) and already used to elucidate the mode of inheritance of EU by multivariate mixed logistic regression (Fritsche *et al.* 2014). All EU diagnoses were finally assessed by one researcher in order to avoid a bias through the first examiner. The dogs were classified based on the more caudally placed ureteral opening into three phenotype classes, namely EU-1 (both ureters in the correct anatomical position with a distance between the more caudal ureteral opening and the vesicourethral junction of at least 1.8 cm (Balogh *et al*. 2015; Rozear & Tidwell 2003)), EU-2 (a distance of the more caudal ureteral opening and the vesicourethral junction between 1.1 and 0.1 cm) and EU-3 (at least one ureteral opening located extravesically in the urethra or genital tract) (Fig. S1). Dogs which had not been examined or for which no conclusive diagnosis was attained, were classified as EU-0. Pedigree information for the dogs was available through an in-house EMD database, merging all EMDs into one single family. We re-estimated the narrow sense heritability (h^2) of EU-3 under a threshold-liability model (Lee *et al.* 2011) using phenotypes of 98 cases (73 males and 25 females) and 151 EU-1 controls (32 males and 119 females) and pedigree

information of 4522 dogs. The analysis included sex as a fixed effect. EU-3 was found to be heritable, with estimates of 0.713 and 0.960 in the 0-1 risk and liability scales, respectively (see supplementary material and methods S1). These findings led us to hypothesize that a major risk locus may underlie EU-3, which could be presumably detected through a genome-wide association study (GWAS).

For the GWAS, genomic DNA was extracted from EDTA blood samples of 381 EMDs with phenotype EU-1 (n=218), EU-2 (n=28) or EU-3 (n=135). Genotyping was performed using the Illumina® CanineHD assay (Illumina Inc., San Diego CA, USA) at GeneSeek (part of Neogen Corporation in Lincoln, USA). This chip contains approximately 173000 single nucleotide polymorphism (SNP) markers distributed throughout the genome with an average density of 70 markers per million base pairs, allowing for a robust within-breed association analysis.
Due to the high selective pressure and inbreeding levels in EMD, our GWAS analysis included dominance and autozygosity effects, apart from additive marker effects (see supplementary material and methods S1). Genome-wide SNP and phenotypic datasets are available via www.animalgenome.org repository and can be accessed using the link https://www.animalgenome.org/repository/pub/UZUR2018.0626/. The family-wise error rate was controlled by adopting a LD-corrected Bonferroni significance level of 0.05/K, where K is the effective number of independent markers. The approach was similar to the simple method (Gao *et al.* 2008), except that K was estimated from the ratio between the total number of markers and the average number of tag-partners per marker ($r2 > 0.3$), instead of the eigenvalues required to explain 99.5% of the variance in the genotype matrix.

Results & Discussion

After scanning all autosomes (Fig. 1), we found six genome-wide significant loci (p-value < 9.65 x 10-5), which are presented in Table 1.
Significant markers were observed in five regions on canine chromosomes (CFA) 3, 17, 27 and 30. Candidate regions mapping to the same chromosome were verified to be independent from each other through LD analysis (Fig. S2). On CFA 3, SNP BICF2P957732 is located near MCTP2, which encodes a transmembrane protein with Ca^{++} binding domains involved in signal transduction or membrane trafficking (Shin *et al.* 2005). Lalani and coworkers (2013) found gross heart anomalies in

Xenopus embryos associated with morpholino knockdown of *MCTP2*. The SNP BICF2P527992 is in an intron of the IQ motif containing GTPase activating protein 1 gene (*IQGAP1*) and presented a significant dominance effect on the investigated trait. *IQGAP1* is an interesting functional candidate gene because it is involved in the regulation of the beta-catenin/GATA3 pathway in *Xenopus* embryos (Goto *et al.* 2013). The beta-catenin/GATA3 pathway is involved in the formation of the ureteric bud, and loss of function of *GATA3* leads to ectopic ureteric bud formation and severe urogenital abnormalities (Grote *et al.* 2008). However, *Iqgap1*-null mice did not show any observable phenotype (Li *et al.* 2000). There is no clear functional candidate gene for the association signal on CFA 17, however, the underlying effects were due to autozygosity in this locus. The most intriguing association signals are on CFA 27. There is one signal at ~1 Mb overlapping with the *HOXC* gene cluster. *HOX* genes are obvious functional candidate genes for phenotypes that involve potential defects in development (Mallo & Alonso 2013). The other association signal at ~23 Mb on CFA 27 is located in an extremely gene poor region upstream of the *SOX5* gene, which encodes another transcription factor involved in development. Coding variants may lead to Lamb-Shaffer syndrome in humans, a neurodevelopmental disorder, sometimes seen in combination with variable skeletal abnormalities (Nesbitt *et al*. 2015) but extremely rarely with urogenital malformations (Lee *et al.* 2013). Therefore, it remains unclear whether *SOX5* is the causative gene underlying this association signal. As the region of this association signal is extremely gene poor, it might have important regulatory functions that are not necessarily restricted to *SOX5* (Ovcharenko *et al.* 2005). The association signal on CFA 30 is located between the *SMAD6* and *SMAD3* genes, encoding again important transcription factors of the TGF-beta signaling pathway that also have a role in development (Macias *et al.* 2015). While our GWAS failed to pick up any candidate genes known from human studies, it is remarkable that 3 of the 5 putative association signals fall near the genes for developmental transcription factors. As the p-values only just exceed the significance threshold, the findings need to be regarded with caution and the associations should be validated in a larger set of animals. In addition, nine genes which were previously shown to be involved in congenital anomalies of the lower urinary tract (CALUT) in humans (reviewed in Rasouly & Lu 2013) were analysed by comparing the genomes of 296 dogs of different breeds and eight EMD genomes

with either EU-1 or EU-3 phenotypes. We did not find any protein-changing variants in these candidate genes (not shown).

Despite the high heritability found for ectopic ureters the association study failed to come up with a single strong association signal. There are at least two possible explanations for such a finding: (1) Despite the high heritability it is possible that many genetic risk loci with small effects are involved in the formation of EU. (2) It also cannot be excluded that a genetic variant, which predisposes the dogs for the formation of ectopic ureters, is fixed in the EMD population and therefore not detectable by the genome-wide association study.

In the latter scenario, modifier genes may still modulate the phenotype despite a unique underlying ectopic ureter genotype, similar to humans (Yosypiv 2012). This assumption is supported by the high average inbreeding coefficient of extant EMDs (Schrack *et al.* 2017), as well as by the much lower prevalence of ectopic ureters in the related Appenzeller mountain dog breed (Bitterli 2011). However, the high heritability seems to contradict this hypothesis. Even if we failed to identify strong candidates known from human studies, the signals seem to be quite clear for the relatively small number of animals, supporting an oligogenic inheritance. However, before using some of those SNPs for marker-assisted selection of breeding EMDs, further research is needed to support this data.

Competing interests

The authors declare that they do not have any competing interests.

Acknowledgements

The authors would like to thank Prof. M. Stoffel and Dr. A. von Rotz (Division of Veterinary Anatomy, Vetsuisse Faculty, University of Bern) for their supporting information about ureter development. The laboratory work was partly performed using the logistics of the Center for Clinical Studies at the Vetsuisse Faculty Zurich, University of Zurich. This study was kindly funded by the Albert Heim Stiftung, the Stiftung für das Wohl des Hundes, as well as the Swiss Federal Food Safety and Veterinary Office. We thank the breeder associations and especially Margret Epple, Herma Cornelese and Max and Gertrud Heller for their support and the private veterinarians for their assistance in acquiring the clinical data.

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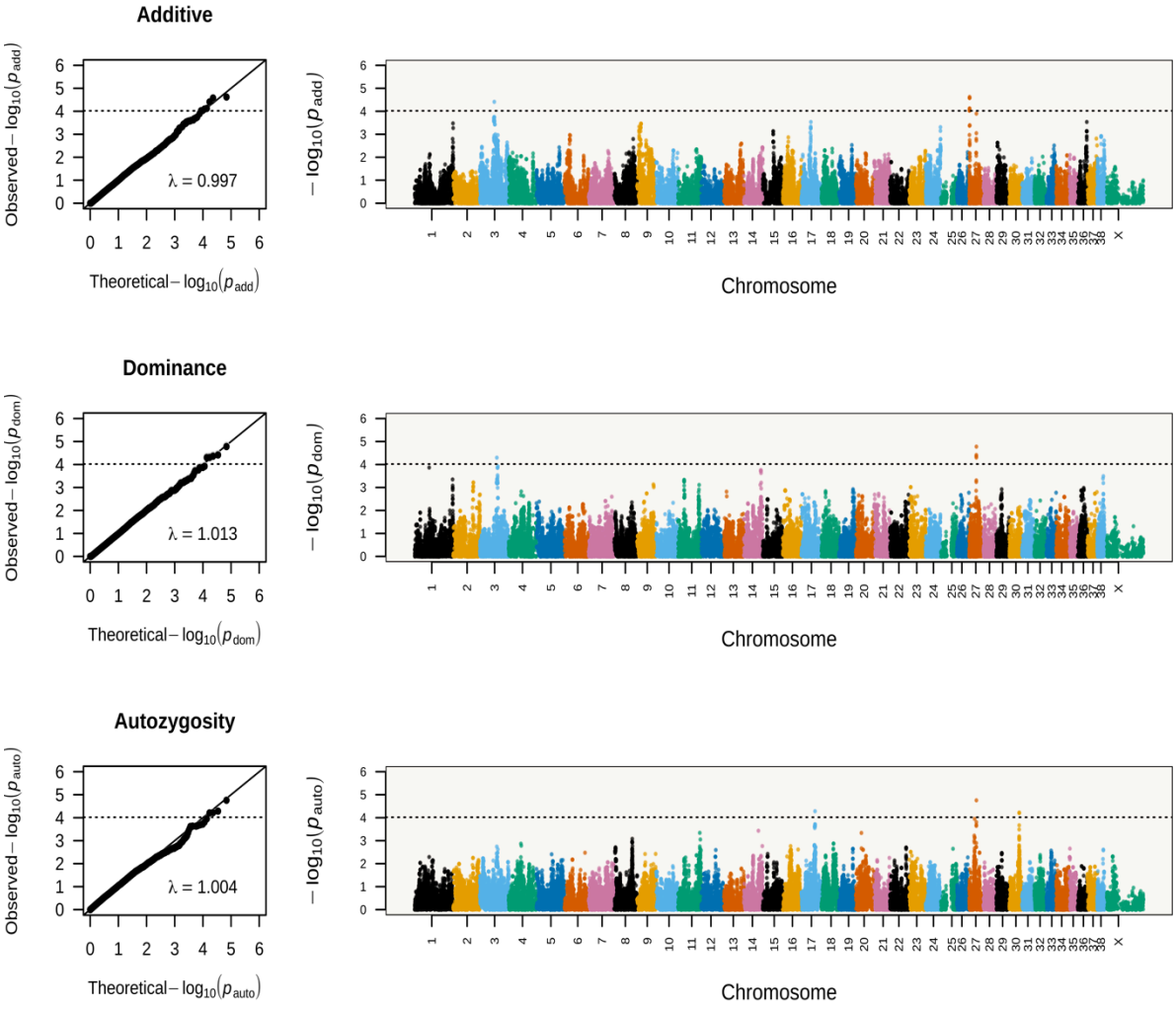

Figure 1. Quantile-quantile and Manhattan plots for additive (add), dominance (dom) and autozygosity (auto) effects. The dashed horizontal line corresponds to the LD-adjusted Bonferroni threshold (p = 9.65 x 10^{-5}).

Table 1. Summary statistics for significant markers associated with ectopic ureters in Entlebucher mountain dogs

CFA[1]	Position[2]	SNP[3]	Position[3]	[4]Alleles	[5]♂ control	[6]♂ case	[7]♀ control	[8]♀ case	[9]Inheritance model	[10]P-value	[11]nearest gene	[12]Distance
3	45655143	BICF2P957732	45655143	A/C	23/9/0	39/27/6	95/22/2	9/13/3	Additive	3.89E-005	MCTP2	187,272
3	53778402	BICF2P527992	53778402	T/C	13/16/3	43/23/7	57/56/6	18/5/2	Dominance	5.05E-005	IQGAP1	0
17	43412582	BICF2P1360326	43412582	C/T	29/3/0	57/15/0	89/29/1	21/3/0	Autozygosity	5.23E-005	CTNNA2	59,764
27	1007730	TIGRP2P347803_rs8943392	1007730	T/C	20/12/0	65/8/0	95/23/1	24/1/0	Additive	9.38E-005	NEF2	0
27	1168218	BICF2S22927985	1168218	G/A	21/11/0	67/6/0	97/21/1	24/1/0	Additive	9.21E-005	HOXC4	41,292
27	1411816	BICF2G630137589	1411816	G/A	13/17/2	54/19/0	73/42/4	19/6/0	Additive	2.63E-005	HOXC13	88,346
27	1415865	BICF2G630137593	1415865	T/C	13/17/2	54/19/0	73/42/4	19/6/0	Additive	2.63E-005	HOXC13	109,704
27	1428628	BICF2G630137612	1428628	G/A	14/16/2	54/19/0	74/41/4	19/6/0	Additive	7.91E-005	HOXC13	12,763
27	1433130	BICF2G630137624	1433130	C/T	13/16/3	52/21/0	71/44/4	19/6/0	Additive	2.38E-005	HOXC13	17,265
27	1437735	BICF2G630137629	1437735	G/A	14/16/2	53/19/0	74/41/4	19/6/0	Additive	7.47E-005	HOXC13	21,870
27	22783765	BICF2G630149192	22783765	C/T	19/12/1	45/25/2	70/46/3	16/5/4	Dominance	4.39E-005	SOX5	73,896
27	22929398	BICF2P1108722	22929398	A/G	18/13/1	43/28/2	70/46/3	16/5/4	Dominance	3.88E-005	lncRNA	0
27	22994047	BICF2P830285	22994047	T/G	18/13/1	41/30/2	69/46/4	16/5/4	Dominance	5.00E-005	lncRNA	0
27	23114194	BICF2P595351	23114194	T/C	18/13/1	40/31/2	69/44/6	16/5/4	Dominance	1.67E-005	lncRNA	0
30	31140902	BICF2P906072	31140902	C/T	22/10/0	46/23/3	78/37/4	16/8/1	Autozygosity	6.06E-005	SMAD3	105,411
30	31236521	BICF2P664860	31236521	A/G	22/10/0	46/24/3	78/37/4	16/8/1	Autozygosity	6.23E-005	SMAD3	9,792

[1] Canine chromosome, [2]SNP position in the corresponding chromosome (CanFam3.1 genome version) Annotation Release 103, [3]SNPs in the Illumina® CanineHD bead chip, [4]minor allele/major allele, [5-8]SNP genotype distribution, [9]gene effect, [10]P-value, [11]gene symbol of the nearest gene of the reported SNP, [12]Distance in bp between SNP and nearest gene (0=SNP within gene).

Appendix S1 Supplementary materials and methods

Estimation of heritability

Phenotypes on the observed 0-1 risk scale were regressed on animal effects through the linear mixed model:

$$\mathbf{y} = \mathbf{X}\mathbf{b} + \mathbf{u} + \mathbf{e}$$

where **y** is the vector of phenotypes, **b** is a vector of unobserved fixed effects (including the overall mean and sex), **X** is a matrix relating phenotypes to **b**, **u** is a vector of unobserved random animal effects, and **e** is a vector of unobserved residual effects. Animal effects were assumed $\mathbf{u}|s_u^2 \sim N(0,\mathbf{A}s_u^2)$, where s_u^2 is the additive genetic variance and **A** is the numerator relationship matrix computed from pedigree data [2]. Residual effects were also assumed normally distributed with mean zero and variance s_e^2. The narrow sense heritability in the observed scale was defined as $h^2_{observed} = s_u^2/(s_u^2 + s_e^2)$. This model was fitted using the restricted maximum likelihood (REML) algorithm implemented in the lme4 v1.1.12 [3] and pedigreemm v0.3.3 [2] packages in R v3.3.2.

We then considered a liability-threshold model [1] where observed disease status results from dichotomization of a standard normal latent variable (i.e., liability). Liabilities of affected animals were assumed to exceed the threshold $t = F^{-1}(1 - p)$, where $F^{-1}(\,.\,)$ is the inverse cumulative function of the standard normal distribution and p is the population prevalence of the disease. Following Fritsche et al. [4], we adopted a prevalence value of p = 0.19, resulting in a threshold of $t = 0.878$. Then, the heritability in the observed scale was converted into heritability in the liability scale through the transformation [1]:

$$h^2_{liability} = h^2_{observed}\, p\,(1 - p)/f(t)^2$$

where $f(\,.\,)$ is the standard normal probability density function. An additional correction for sampling bias was adopted, namely $h^2_{liability}$ was multiplied by $p(1 - p)/[p(1 - p)]$, where p is the sample proportion of cases.

GWA analysis

Let $\mathbf{V} = \mathbf{A}s_u^2 + \mathbf{I}s_e^2$, where **A** is the numerator relationship matrix and s_u^2 and s_e^2 are variance components estimated from the model presented in the previous section. Now let $\mathbf{b} = [\mathrm{m}\ s\ \mathrm{a}\ d\ i]^T$, where m is the intercept, *s* is the effect of sex, a is the allele substitution effect (i.e., additive effect), *d* is the dominance effect, and *i* is the inbreeding (i.e., autozygosity) effect. Also, define **X** = [**1 k z w h**], where **1** is a vector of ones, **k** is an indicator vector for sex, and **z**, **w** and **h** are design vectors for additive, dominance and autozygosity effects, respectively. Considering alleles 1 and 2 with frequencies *p* and $q = 1 - p$, respectively, vectors **z**, **w** and **h** were encoded as:

z = $0 - 2p$, for genotype 22
$1 - 2\mathrm{p}$, for genotype 12/21
$2 - 2p$, for genotype 11

w = $-2p^2$, for genotype 22
$2pq$, for genotype 12/21
$-2q^2$, for genotype 11

h = 1, for autozygous genotype
0, for non-autozygous genotype

Codes in **z** and **w** followed classical parameterization of genotypic values under additive and dominant gene action [5,6], whereas autozygosity status was determined based on the presence of a run of homozygosity (ROH) encompassing the genotype (see the next section). For markers mapping to the X chromosome, dosage compensation was taken into account through replacement of male hemyzygous genotypes 1 and 2 by 11 and 22, respectively.

Fixed effects were estimated as:

$$\mathbf{b} = (\mathbf{X}^{\mathrm{T}}\mathbf{V}^{-1}\mathbf{X})^{-1}\mathbf{X}^{\mathrm{T}}\mathbf{V}^{-1}\mathbf{y}$$

with variance:

$$\mathrm{VAR}(\mathbf{b}) = \mathrm{diag}[(\mathbf{X}^{\mathrm{T}}\mathbf{V}^{-1}\mathbf{X})^{-1}]$$

Marker effects were tested for significance via the chi-squared statistic $b_j^2 / \mathrm{VAR}(b_j)$, where b_j is a, *d* or *i*, and $\mathrm{VAR}(b_j)$ is the variance of b_j. Prior to *p*-value calculation, all statistics were divided by the inflation factor estimated from regression of observed statistics onto theoretical quantiles. In summary, our GWA analysis was very similar to other widely used linear mixed model approaches [7–9], except that our procedure built on the conventional additive model to accommodate dominance and inbreeding effects (which are not usually implemented in GWA tools), and that confounding due to family structure was controlled by the use of pedigree data instead of genotypic data.

A given marker effect was declared significant if its *p*-value was smaller than the Bonferroni-corrected significance level 0.05/*K*, where *K* is the effective number of independent markers. Quantity *K* was estimated as *N*/*T*, where *N* is the total number of markers and *T* is the average number of tag-partners per marker with linkage disequilibrium $r^2 > 0.3$. Numbers of tag-partners were obtained using the --show-tags algorithm in PLINK v1.9 [10,11]. The search for tag-partners was constrained within the same chromosome. In this study, we observed N = 68,842 and *T* = 133 (i.e., each marker was tagged by other 133 markers on average), resulting in *K* = 518 and a significance threshold of 9.65×10^{-5}.

Detection of runs of homozygosity

In order to detect ROH segments, we developed a procedure that auto-tunes the parameters of the heuristic algorithm in PLINK v1.9 [10,11] according to ROH size. Briefly, instead of using a fixed set of parameters for detecting ROH of all lengths, parameter values were dynamically adjusted as ROH size increased.

This is desirable given long ROH tend to be scattered into smaller segments when detection parameters are not corrected for ROH size [12]. A more detailed description of this procedure is provided below. First, we defined the following fixed parameters:

mdist. Vector of intermarker distances (in kbp).
gensize. Marked genome size (in kbp), calculated from summation of *mdist*.
max(mdist). Largest value of *mdist* (in kbp).
chrsize. Size of the chromosome exhibiting the largest value of *mdist* (in kbp).
gapratio. Ratio between *max(mdist)* and *chrsize*.
e*rror*. Genotyping error rate (assumed 1%).
callrate. Baseline call rate (assumed 95%).
density. Minimum marker density in a ROH (kbp/marker), obtained as the 99th percentile of *mdist*.

and the following variable parameters:

rohmin. Minimum ROH size (in kbp).
rohmax. Maximum ROH size (in kbp).
minsnp. Minimum number of markers in a ROH, calculated as *rohmin*/*density*.
gap. Maximum allowed gap size in a ROH (in kbp), defined as *rohmin*gapratio*.
het. Maximum number of heterozygote genotypes allowed, defined as *error*minsnp*.
miss. Maximum number of missing genotypes allowed, defined as (1 – *callrate*)**minsnp*.

We then performed detection of ROH independently for the following pairs of *rohmin* and *rohmax* (represented here in Mbp): (1, 2], (2, 4], (4, 8], (8, 16] and (16, *gensize*]. For each of these pairs, PLINK v1.9 was ran with the following settings:

--homozyg-window-snp [*minsnp*]
--homozyg-window-het [*het*]
--homozyg-window-missing [*miss*]
--homozyg-window-threshold [0.05]
--homozyg-snp [*minsnp*]

--homozyg-kb [*rohmin*]
--homozyg-density [*density*]
--homozyg-gap [*gap*]
--homozyg-het [*het*]

The resulting segments in each analysis were then pruned to exclude ROH larger than *rohmax*. After all analyses were completed, overlapping segments were merged with BEDTools v2.25.0 [13]. **Table 1** presents the parameter values used in each analysis. Prior to ROH detection, genotypes were filtered for call rate > 0.95 and GenTrain score > 0.8. To avoid false negative ROH, no minor allele frequency filter was used, which resulted in 136,846 markers included for ROH identification.

Table 1. Parameter values for the detection or runs of homozygosity according to segment size.

Segment size (in Mbp)	Minimum number of markers	Maximum number of heterozygous genotypes	Maximum number of missing genotypes	Maximum gap size (in kbp)	Minimum marker density (in kbp/marker)
(1, 2]	17	0	1	45	60
(2, 4]	33	0	2	91	60
(4, 8]	67	1	3	182	60
(8, 16]	133	1	7	363	60
>16	266	3	13	726	60

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Figure S1. Dorsal view of the bladder of a male dog. For an easier view spermatic ducts are not drawn. Dotted lines show borders for the classification of ectopic ureters: EU-1 (normal) openings into the trigonum; EU-2 (intra-vesicular ectopic ureters) openings into the bladder neck; EU-3 (extra-vesicular ectopic ureters) openings into urethra or genital organs.

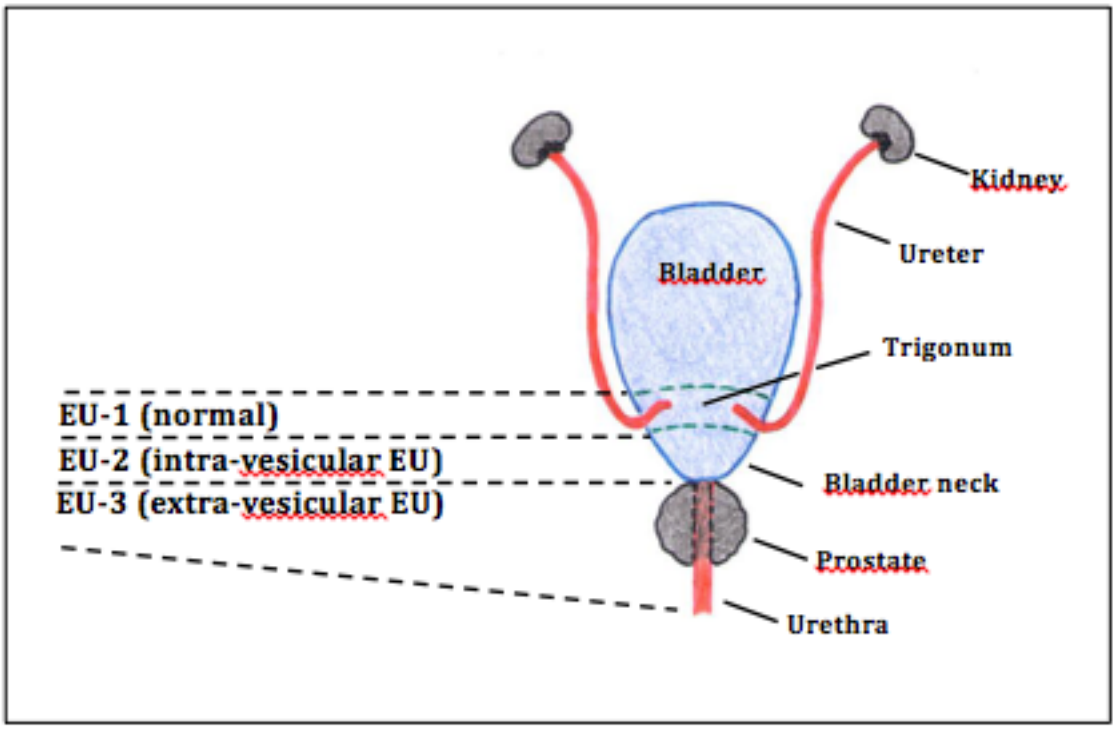
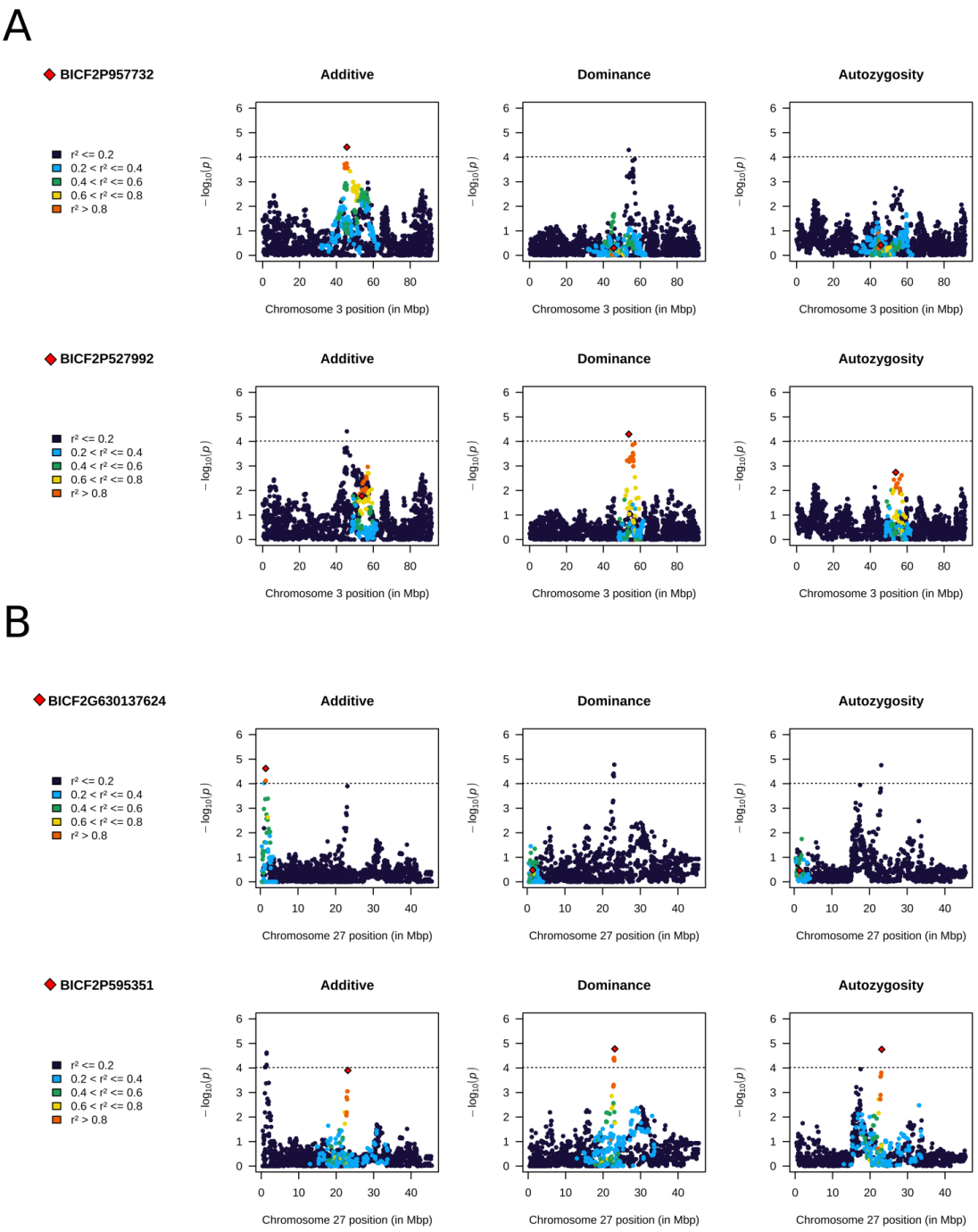


Figure S2. Regional association plots of chromosomes exhibiting more than one association signal. Chromosomes 3 (A) and 27 (B) present two signals each that are revealed to be independent from each other when the leading markers (red diamonds) are inspected for linkage disequilibrium (r^2) with nearby SNPs.



Danksagung

Während meiner Zeit als Doktorandin und Assistenzärztin auf der Abteilung für Kleintierreproduktion durfte ich auf die Hilfe vieler Menschen zählen. Daher möchte ich mich an dieser Stelle ganz herzlich bei allen bedanken, die zum Gelingen meiner Arbeit beigetragen haben.

Besonderer Dank gilt meiner Referentin Prof. Dr. Iris M. Reichler, welche enorm viel Zeit in diese Arbeit gesteckt hat, immer wieder neue Ansätze einbrachte und jederzeit ein offenes Ohr für mich hatte. Ohne sie gäbe es diese Dissertation nicht. Zudem habe ich auch im klinischen Bereich sehr viel von ihr gelernt.

Des Weiteren möchte ich mich bei PD Dr. Claude Schelling bedanken. Auch er hat sehr viel Zeit in dieses Projekt investiert und war mir insbesondere beim Erstellen des Publikationsmanuskripts eine grosse Hilfe.

Bei statistischen Problemen oder Fragen zur Genetik konnte ich stets auf die Unterstützung von Prof. Dr. sc. techn. Gaudenz Dolf und Prof. Dr. Tosso Leeb von der Vetsuisse-Fakultät Bern zählen. Auch ihnen gilt mein Dank.

Natürlich auch all meinen Mitautoren der publizierten Version: Herzlichen Dank!

Und schliesslich wäre diese Arbeit ohne die Mitarbeit der Zuchtwarte, der Hundebesitzer, sowie der untersuchenden Tierärzte aus der Schweiz, Deutschland und Österreich nicht möglich gewesen. Daher danke ich auch ihnen für ihr Interesse und ihre Unterstützung.

Ich hatte als Assistenzärztin auf der Kleintierreproduktion ein Team, mit dem ich wunderbare, unvergessliche, meist lustige und ab und zu auch schwere Momente erleben durfte. Dieses Team ist massgeblich daran beteiligt, dass ich mich stets im Guten ans Tierspital erinnern werde. Vielen Dank, ich freue mich auf weitere tolle Momente zusammen!

Last, but certainly not least danke ich meinen Eltern, ohne die ich meinen Weg nicht so hätte gehen können, wie ich es getan habe. Sie stehen mir immer mit Rat und Tat zur Seite und sind meine grösste Stütze. Dasselbe gilt auch für meine Schwester, die alles mit mir teilt, stets für mich da ist und sich über jeden erreichten Meilenstein riesig für mich freut. Grazie, merci, ihr seid die Besten!

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